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by G. D. Khudadov

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A METHOD OF TAGGING INSECTS BY GIVING THEM RADIOACTIVE ISOTOPES WITH FOOD

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A large number of infectious diseases are transferred by insects, acting as specific and non-specific carriers. Considerable funds and materials as well as the efforts of a large number of workers, are being expended in the control of arthropoda, but these expenditures do not always ensure effective and stable epidemic results. The success in the prophylaxis and control of infectious diseases, which are transferred by means of carriers of the infectious agent, can be achieved only if the physiological and ecological peculiarities of insects are taken into account.

A valuable method in the study of the area and rate of propagation of arthropoda, as well as a number of other problems associated with them, is the tagging of insects and ticks. In order to tag the insects, a number of authors employed radioactive isototpes in administering food to them. Thus, Lindquist, Yates, and Hoffman (1951) included P^{32} in drinking water with sugar and gave this radioactive food to hungry insects. The specific radioactivity of food was 1.6 microcuries per ml. It turned out that with such a method of tagging about 90 percent of the flies became radioactive and recorded from 200 to 5,000 imp/min.

The same composition of food for tagging flies with radio-phosphorus (3.9 microcuries per ml) was successfully employed by Roan (1950).

Pimontel and Fay (1955) used P^{32} in the form of a

mono-derivative of sodium phosphate for the tagging of drosophilidae. Food containing a radioisotope in concentration of two microcuries per ml consisted of bananas and yeast. As shown by experiments, the one-to-eight-hour exposure of food proved insufficient, while a 24-hour exposure gave satisfactory results. The radioactivity of females ranged from 240 to 2,100 imp/min and that of males, from 20 to 250 imp/min. After a few days, one could not elicit any radioactivity in males, whereas the females recorded from 235 to 1,350 imp/min on the counter.

McLeod and Donnelli (1957) used pure water or a sugar solution for mass tagging of flies with radiophosphorus. The exposure of the food of flies lasted from 48 to 72 hours. The radioisotope concentration in food fluctuated between 13 and 35 microcuries per ml of food. When a radioactive medium containing 35 microcuries/ml was employed, the radioactivity of flies equalled on the average 598 imp/sec (from 50 to 1,000). The used concentrations enabled the authors to detect tagged flies for a period of several weeks.

B. L. Shura-Bura (1955), in studying the migration of flies from garbage dumps to residential dwellings, placed at the dum sites bowls of bait, consisting of fermenting yeast, sugar, and bran. The P^{32} concentration in the food amounted to five million imp/sec per ml (i.e., 126 microcuries/ml). In preliminary tests he established that upon single feeding of house flies with an exact dose of one to two mg of P^{32} solution of 10 million imp/min activity per ml (i.e., 4.2 microcuries/ml), the radioactivity could be detected in flies during the first four to five days, and in small quantities up to the 11th day.

N. B, Il'inskaya and A. S. Troshin (1954) gave the flies food of one microcure P^{32} per ml specific radioactivity. 24 hours after feeding, the house flies showed 9,800 imp/min on the average, and 16 days later -- 200 imp/min. Foott (1954) established that female cabage flies (Hylemyia brassicae) irradiated notably larger quantities of impulses than males; the author ascribes this difference to the large size of females, and, consequently, the consumption of a greater quantity of radioactive food with P^{32}.

Radioactive phosphorus was also employed in tagging other insects. Thus. Lüdicke (1954) tagged black and reddish roaches by given them P^{32} with their food. Kuper, Pels (1953) tagged Aedes mosquitoes by giving them a sugar solution with radiophosphorus in concentration from 0.2 to 3 microcuries per ml after they had fed on [the blood of] birds.

A somewhat different method of mosquito tagging was employed by Hassett and Jenkins (1949), where the mosquitoes were fed on flowers which had been immersed in a radiophosphorus-containing solution. Yates, Gjullin, Lindquist (1951) administered intraperitoneally to rats 376 microcuries of P^{32}, and the following day allowed mosquitoes to feed on them. After a selective checking following the feeding, the following radioactivity was observed in the mosquitoes: 740, 893, 397, 881, 776, and 544 imp/min.

Besides P^{32}, the authors employed other radioactive isotopes. Cunliff (1952) in tagging roaches, fed them food containing chlorine-36 in the form of sodium chloride. Ring and Layne (1953) tagged coleoptera Conotrachelus nenuphar Hbst., harmful to prunes, with strontium-89. This method, according to the authors, proved to be of little value when cobalt-60 and iodine-131 were mployed, and mildly effective upon the use of zinc-65.

Nixon and Ribbands (1953) tagged bees with P^{32} and carbon-14. The authors arrived at the conclusion that carbon-14, though a specific agent for tagging, is of little use in this particular work, due to the difficulties of the determination and measuring of its very weak beta radiation, and they discontinued using it.

Hamilton (1935) fed plant lice with a substrate containing radioactive polonium. Hinton (1954) used barium-140 and carbon -14 for the purpose of studying their distribution in the organism of insects.

N. Ye. Il'inskaya and A. S. Troshin tagged house flies by feeding them a solution of glucose to which one microcurie per ml of calcium-45 had been added. They obtained tagged files which recorded an average of about 1,600 imp/min during the first day following cessation of tagging, and on the 10th day -- less than 200 imp/min. The

authors arrived at the conclusion that radioactive calcium is rapidly eliminated from the organism of flies.

Quarterman, Mathis, Kilpatrick (1954) employed phosphorus-32, calcium-45, and iodine-131 for tagging flies. The radioactive calcium and iodine proved to be of little use because calcium in the form of calcium chloride caused the death of almost 50 percent of the flies, and iodine also led to considerable fly fatality, especially of males.

Thus, the researchers used a number of radioactive isotopes for tagging insects; some of these were found to be useful for this purpose, and others proved unsuitable. At the same time none of the researchers developed a precise method of tagging.

We set ourselves the task of determining which of the radioactive isotopes could be used for tagging purposes, and to work out in detail a method of tagging insects by administering the isotope with their food. With this in view we used a whole group of radioisotopes (Table 1) which have various half-life periods and possess only beta and gamma radiation, as well as mixed beta-gamma radiation. Chemical compounds easily soluble in water were used in the experiments.

Of the radioisotopes used, P^{32} was acknowledged by the predominant majority of authors to be most suitable for tagging purposes. In our studies it also resealed itself as an isotope useful for tagging purposes, and we employed it as a standard in working out methods of tagging for all problems connected with it.

House flies and reddish roaches were chosen by us as convenient and easily available objects for the development of the outlined problems. The tagging of house flies was conducted in gas enclosures 20 x 20 x 20 cm in size. The food was poured in Petri dishes into which a thin layer of rubber sponge was placed, in order to prevent drowning of flies in the fluid. From 250 to 400 flies were placed in each breeding place. After the feeding of insects had been completed, the food containing radioactive substance was replaced by food without an isotope, and the measuring of the radioactivity of flies with counters was

Table 1

Radioactive isotopes and their chemical compounds employed in the experiments

Isotope	Half-life period in days	Character of radiation	Energy of radiation (Mev)		
			of particles	of gamma rays	
Phosphorus-32	14,3	β^-	1,701	none	Na_2HPO_4 K_2HPO_4
Calcium-45	152	β^-	0,25	none	$CaCl_2$
Iron-59	45,1	β^- γ	0,460 (50%) 0,257 (50%)	1,295 1,097	$Fe_2(SO_4)_3$
Zinc-65	250	β^-(2,5%) K (97,5%) γ^-	0,325 (45%)	1,118	$ZnCl_2$
Strontium-89	53	β^-	1,46	none	$SrCl_2$
Yttrium-91	61	β^- γ	1,537	1,2	YCl_3
Cadmium-115	43	β^- γ	1,6 (98%) 0,7 (2%) 0,3 (weak)	1,28 0,96 0,50 0,46	$CdCl_2$
Iodine-131	8,14	β^- γ	0,815 (0,7%) 0,608 (87,2%) 0,335 (9,3%) 0,250 (2,8%)	0,722 0,284 0,637 0,163 0,364 0,080	NaJ KJ
Barium-140	12,8	β^-	1,022 (60%) 0,480 (40%) 0,26 (10%)	0,537 0,304 0,162	$BaCl_2$
Lanthanum	40 hours	γ	1,67 (20%) 1,32 (70%)	0,132 5,0296	

Table 2

Dependence of radioactivity of flies on the exposure of their food

Food exposure in hours	Average number of imp/min of flies on days following the end of feeding			Percentage of flies
	1	2	3	
1	1277	716	550	60
2	1317	859	728	82
3	1586	910	800	88
24	3418	2063	1781	100

Table 3

Dependence of radioactivity of room flies on the concentration of phosphorus-32 in the food

Concentration of P^{32} in food in microcuries/ml	Days after feeding				Average extensive index
	1		3		
	imp/min	Extensive index	imp/min	Extensive index	
0,25	972	28,5	548	30,3	29,4
0,50	1582	46,2	929	52,2	49,2
1,00	3418	100	1781	100	100
1,50	4513	132	2555	143,6	137,8
2,00	7400	216,4	3782	212,5	214,4
3,00	10803	315,9	4770	268,0	291,9
4,00	15976	467,1	7676	431,2	449,1
5,00	20060	586,4	7970	447,8	517,1

started; these determinations were carried out on alternate days for 19 days.

Our first task was to determine the food exposure of house flies needed to obtain 100 percent tagging of insects. The experiments were conducted with a food exposure equal to 1, 2, 3 and 24 hours. The flies were given food with phosphorus-32 of a specific gravity of one microcurie/ml. The data obtained are cited in Table 2.

As is seen in Table 2, the one-hour exposure of food ensures tagging of only 60 percent of flies, a two- and three-hour duration of feeding -- 82 percent and 86 percent respectively, and a 24-hour exposure -- 100 percent [tagging] of radioactive flies. Besides, with the lengthening of the food exposure the radioactivity of flies increased correspondingly. On the basis of obtained data, we employed in our subsequent work a 24-hour exposure in tagging house flies by the food method. After that we proceeded to establish the food composition which would ensure the best results in tagging flies.

Radioactive phosphorus-32 was added to food at the ratio of one microcurie per ml of food. As a result, it was ascertained that the best results were ensured by the following food composition: three parts milk and one part of 10 percent sugar water.

These data were verified with other isotopes.

This composition of the selected food proved to be the best in the case of all isotopes, with the exception of radioactive Fe-59; in this case we had to use sugar water alone.

To ascertain the relation between the radioisotope concentration in the food and the radioactivity of flies following feeding, we tested eight P^{32} concentrations in food from 0.25 to 5 microcuries/ml (Table 3). The radioactivity of flies obtained after their tagging with food containing radiophosphorus in one microcurie/ml concentration was accepted as 100 percent.

As is seen from Table 3, there is a direct relation between the concentration of radioisotope in food and the radioactivity of flies. Thus, upon a two-fold increase of the quantitity of radiophosphorus in food, the radioactivity of flies increases approximately two-fold, etc. (Fig. 1).

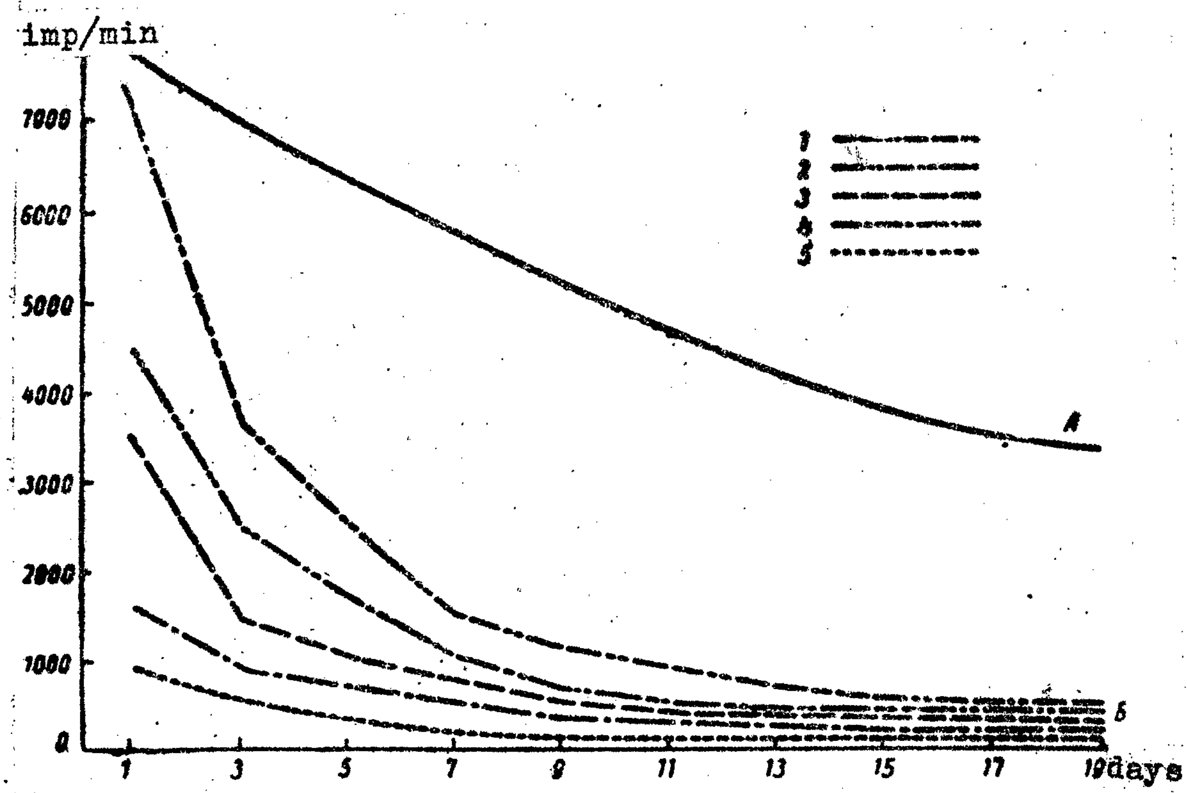

Fig. 1. Radioactivity of house flies tagged with P^{32} (Na_2HPO_4) by the feeding method.

A -- theoretical curve of the reduction of radioactivity; B -- radioactivity curves of flies fed on P^{32}; 1 -- 2.0 microcuries/ml; 2 -- 1.5 microcuries/ml; 3 -- 1.0 microcurie/ml; 4 -- 0.5 microcurie/ml; 5 -- 0.25 microcurie/ml.

The radioactivity of females proved to be twice as high as that of males (Fig. 2), which is explained by the approximately two times as high weight of female house flies (the average weight of a female is 26.7 ± 0.59 mg, of a male -- 13.6 ± 0.09 mg).

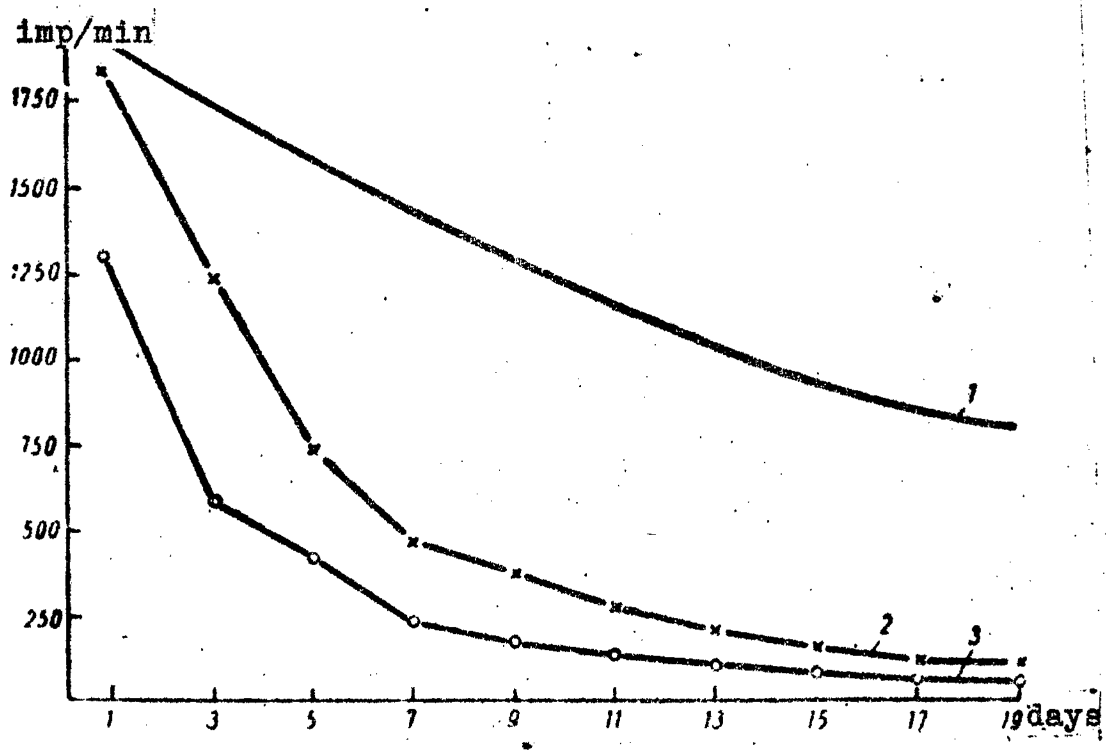

Fig. 2. Comparative radioactivity of male and female house flies tagged with P^{32} (Na_2HPO_4) by the feeding method (isotope concentration in food -- 0.5 microcurie/ml).

Radioactivity reduction curves: 1 -- theoretical; 2 -- of females; 3 -- of males.

In connection with the fact that females possess twice as much radioactivity as males, a question arose regarding the range of the mean arithmetical radioactivity of house flies at various days following cessation of tagging. In conducting these determinations we used an equal number of males and females. After making relevant calculations, we obtained a variation-coefficient equal to 30.6 percent for the first day, and 39.2 percent for the ninth day.

Considering that the radioactivity of female house

flies is nearly twice as high as that of males, the obtained variation coefficients indicate that one can use mean arithmetic data of radioactivity of flies at various days following termination of their tagging by the feeding method.

In studying the radioactivity reduction curve of house flies tagged with phosphorus-32 by the above-described method, we established that at a concentration of one microcurie/ml of the isotope in food, the radioactivity is reduced approximately twofold on the third day following feeding; subsequently, the radioactivity reduction proceeds at a more retarded rate and on the 19th day represents only four percent of the original figure (Fig. 1 and Table 4).

Table 4

Radioactivity of house flies tagged with P^{32} (the isotope concentration in food is one microcurie/ml)

Days following cessation of food exposure	1	3	5	7	9	11	13	15	17	19
imp/min	3418	1781	1025	811	446	316	286	261	182	156

It has been established that the radioactivity in males proceeds at a somewhat faster rate than in females -- on the average, approximately, 1.5 times faster.

It is necessary to note here that results obtained with the use of P^{32} in double derivative potassium phosphate and double derivative sodium-phosphate chemical combinations are identical.

Having clarified all above-mentioned problems with our "standard" phosphorus-32, we carried out a number of studies to establish the possibility of using other isotopes for these purposes. It was necessary to establish criteria which would enable us to determine the suitability of various isotopes for tagging purposes .

With this in view we selected at the first stage the following isotopes: calcium-45 which possesses only

beta radiation, iodine 131 and Fe-59 which possess beta and gamma radiations, and zinc-65 which possesses only gamma radiation. These isotopes were selected because they have various half-life periods, as well as various periods of radiation (Table 1). In addition, the radioisotopes which we had selected, and which possess beta radiation, are also characterized by various mean energies of higher energy levels of beta-spectra. The water-soluble chemical compounds of these isotopes, as well as of others, were employed.

The attempts to tag house flies with iodine-131, iron-59, and calcium-45 food of one microcurie/ml radioisotope concentration were not successful because no clearly recorded data could be obtained. by increasing the isotope concentration in food to a dose of 50 microcuries/ml of iodine-131 and calcium-45, precise data were obtained; iron in this concentration proved toxic to flies, and we did not use concentrations over 20 microcuries/ml. The obtained results are shown in Table 5; for the convenience of comparison the data obtained in feeding iron-59 to the flies were extrapolated, since we had established and demonstrated that upon increased concentration of isotopes in food the radioactivity of flies increases proportionally.

Table 5

Radioactivity of house flies tagged with various radioisotopes by the feeding method
(isotope concentration in food - 50 microcuries/ml)

Radioisotope	imp/min on days following cessarion of food exposure							
	1	3	5	7	9	11	13	15
Iodine-131. .	2040	1180	991	803	650	410	360	290
Iron-59 . . .	800	500	440	406	325	290	225	175
Calcium-45. .	365	213	135	61	41	23	12	10

As seen from Table 5, the radioactivity of flies fed on various isotopes is reduced at various rates. Thus, it is reduced the fastest when calcium-45 is used, and the slowest when iron-59 is employed; iodine-131 occupies the intermediate position. This phenomenon is connected in the first place with the rate of elimination from insect organisms of the chemical compound in which the given isotope is present, and in the second place -- with the natural reduction of the isotope radioactivity as the result of its inherent disintegration.

A study of the elimination rate of various radioactive isotopes from the organism of house flies showed (Table 6) that calcium-45 is eliminated most rapidly; on the fifth day there remain only three percent of the administered isotope in the organism of flies, though its half-life period is longer than in other isotopes. Iodine-131 which has a half-life period of 8.14 days is eliminated from the organism more slowly than any other / isotope /, and 47 percent of it is retained in the organism of flies toward the 15th day.

Table 6

Elimination of radioactive isotopes from the organism of house flies, together with the calculation of the natural disintegration / rate / of the isotopes

Radioisotope	Half-life period in days	Percentages of radioactivity in relation to the first day on days following cessation to food exposure							
		1	3	5	7	9	11	13	15
Iodine-131	8,14	100	68	68	66	63	47	48	47
Phosphorus-32	14,3	100	58	36	32	19	15	15	15
Iron-59	45,1	100	64	59	56	46	42	34	27
Calcium-45	152	100	59	38	17	12	7	4	3

It is necessary to emphasize the point that data cited in Table 6 reflect only the elimination of the radioisotope from the organism of insects; a corresponding correction was made in regard to its / rate of / natural disintegration.

On the basis of data cited in Table 6 one can concur with certain authors who assert that calcium-45 is

unsuitable for tagging purposes, on account of its rapid elimination from the organism of insects.

In the final analysis we are interested in the general reduction of radioactivity, which depends on the elimination of the radioactive isotope from the organism of insects, as well as on its half-life period.

It remains obscure, however, why flies given food of 50 microcuries/ml concentration of calcium-45, iron -59, or iodine-131 (Table 5) and one microcurie/ml of phosphorus-32 (Table 4) emit a different number of impulses following cessation of the food exposure.

In analyzing this problem, it becomes obvious that there is a different mean energy of beta particles in the four radioactive isotopes (Table 1). Upon comparing the data it becomes clear that the higher the mean energy of the higher energy levels of beta particles, the greater is the number of impulses recorded by counters of house flies fed on a given isotope. The beta particles of calcium-45 possess the least power (Table 5), and the number of recorded imp/min from flies fed on this isotope is the lowest; the energy of beta particles of iron-59 is greater than that of calcium-45, and the recording of imp/min from flies tagged with this isotope is higher, etc. Here we see an intermittent transition of quantitative changes into qualitative. The physical essence of this phenomenon consists of the fact that the length of the run of beta particles in matter depends on their energy. In Table 7 (according to V. A. Patrov) are cited

Table 7

The length of run of beta particles depending on the maximal energy

Maximal energy of the Mev beta spectrum	0,1	0,3	0,6	0,9	1,2	1,5	1,8
Air (cm)	16	97	240	440	600	790	600
Water (mm).	0,13	0,8	2,0	3,5	5,2	6,7	8,0
Aluminum (mm). . .	0,06	0,36	0,91	1,6	2,1	2,8	3,4

the data of the length of the run of beta particles in various media, depending on their maximal energy. One can, with a certain allowance, compare the length of run of beta particles in tissues to the run of these particles in water. As seen from the table, the length of run of these particles is directly dependent on their energy and it does not increase proportionally to the increase of their energy, but by degrees.

In tagging insects with radioactive isotopes administered to them with food, the radioisotope remains within the organism of the insects, because their surface contamination is slight, as we had ascertained. Therefore, before the beta particle enters the recording counter it has to pass through a layer of the insect's tissues, and afterwards through a layer of air between the insect and the counter. Since the tissue lining is only 15 mm distant from the counter, and since the run of beta particles in the air is large (160 mm for particles with energy equal to 0.1 Mev), the absorption of the particles by the air [layer] between the insect and the counter can be disregarded. Besides, the counters do not record all beta particles, but only those which possess a definite minimum and maximum energy. This phenomenon was taken into account by us in the selection of counters for recording particles of various energy.

It remains for us to examine the problem of the absorption of beta particles upon their passage through the tissues of insect organisms. In order to clarify this problem we conducted the following experiments. The radioactivity of flies tagged by the food method was determined, the flies were then very finely ground (i.e., the absorbing action of the tissues was reduced to a certain extent), and the radioactivity was again determined (Fig. 3); the recorded number of imp/min from whole flies was taken as 100. As is seen from Fig. 3, the increase of the number of recorded impulses varies inversely with the energy of the particles, i.e., the higher the energy of the particles the less is the increase of imp/min in a ground fly as compared to a whole fly. Since female house flies weigh more than males, the increase of recorded imp/min in ground female house

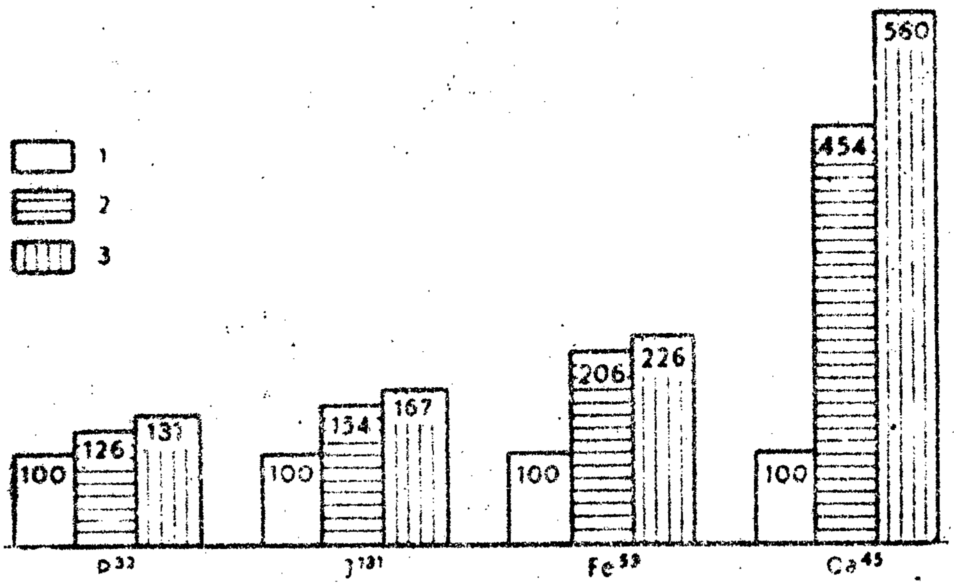

Fig. 3. Increase of recorded imp/min from ground female and male house flies as compared to whole insects (depending on the energy of beta particle isotopes):

1 -- recorded imp/min from whole flies (taken as 100);
2 -- recorded imp/min from ground male flies;
3 -- the same from females.

flies, compared to whole flies -- as is seen in Fig. 3 -- is greater than that of male flies. This leads to the very important conclusion that isotopes possessing hard beta radiation are best suited for tagging insects by means of administering the radioisotope with food.

The attempt to utilize zinc-65, which possesses gamma radiation, i.e., radiation of high penetration, proved unsuccessful (Table 8). This is due to the fact that while the effectiveness of counters in the case of beta particles approaches 100 percent, it is much lower for gamma particles and fluctuates within the limits of 0.1 to 1 percent (K. K. Aglintsev, 1957). The flies were tagged with food containing zinc-65 in 24 microcuries/ml concentrations; higher concentrations proved

to be toxic to insects.

As is seen from Table 8, the recorded number of imp/min from flies tagged with zinc-65 was small, and, in addition, a rapid reduction of the radioactivity of flies took place.

Table 8

Radioactivity of house flies tagged with zinc-65 by the food method (isotope concentration in food -- 25 microcuries/ml)

Days following cessation of food	1	3	5	7	9
imp/min.	318	51	38	27	17

To verify the data obtained in regard to the fact that radioactive isotopes with hard beta radiation are suitable for tagging insects by adding the isotopes to their food, we carried out experiments with the following radioactive isotopes (the mean energy of beta-spectra peak energy levels is shown in parenthesis): cadmium-115 (1.58 Mev); yttrium-91 (1.564 Mev); strontium-89 (1.463 Mev), and barium-140 (0.805 Mev). The data obtained are shown in Table 9.

Table 9

Radioactivity of house flies tagged with radioisotopes by the food method (concentration of isotopes in food -- three microcuries/ml)

Radioisotope	imp/min on days following cessation of food									
	1	3	5	7	9	11	13	15	17	19
Yttrium-91 . .	5567	459	180	170	110	108	86	68	60	51
Strontium-89	7034	1364	599	381	298	262	231	220	214	188
Barium-140 . .	7037	1098	559	343	115	133	110	99	89	63

As is seen from Table 9, the radioactivity of flies is sharply reduced on the third day following cessation of food exposure, and is reduced rather slowly after that. The recorded number of imp/min from flies fed on food containing radioisotope concentration equal to three microcuries/ml permits us to consider these isotopes suitable for tagging purposes.

Table 10

Radioactivity of insects tagged with phosphorus-32 by the food method (concentration of the isotope in food — one microcurie/ml)

Species of insects	Composition of food	imp/min following cessation of food exposure		
		1	3	5
Calliphora erytrocephala	three parts milk + one part of 10% sugar water	3775	1780	778
Culex pipiens	piece of cotton soaked in water	54	27	—
Blattella germanica	rye biscuits	1432	720	—

Radioactive isotopes in the employed water soluble chemical compounds proved to be non-toxic to insects, with the exception of cadmium-115 (in a one microcurie/ml dose). The advantage of the selected radioactive isotopes possessing beta radiation with a mean energy of particles of higher energy levels equal to an order or 1,800 Mev (barium-140) and higher consists also of the fact that flies tagged by them retain their radioactivity throughout the course of the experiment. It is necessary to note that some of the house flies tagged with calcium-45 did not emit impulses on the third day; when iron-59 was used, 50 percent of the flies proved to be untagged on the ninth day; the same can be said of iodine-131 when the concentration in food of this radioisotope was equal to 20 microcuries/ml) and of zinc-65 during whose use up to 24 percent of the flies proved to be untagged even during the first few days.

To verify the suitability of tagging other

insects by the method thus formulated, we carried out experiments with Culex pipiens mosquitoes, Calliphora erytrocephala flies and reddish roaches. These tests showed that upon giving them food containing radiophosphorous for 24 hours (for roaches -- 48 hours), all specimens proved to be tagged. The data cited in Table 10 show that their radioactivity is reduced along the same curve as in the tagging of house flies.

Conclusions

1. For purposes of tagging insects by adding a radioisotope to their food, those radioisotopes are suitable which possess beta radiation with a mean energy of higher energy levels of beta-spectra of an 0.8 Mev order and higher, and which have a half-life of not less than one week.

2. Upon an increase of radioisotope concentration in food, there is a proportional increase of the radioactivity of the insects which have been fed on it.

3. To carry out experiments with tagged insects for a period from 10 to 20 days, one can tag them by giving them food containing a radioisotope in a concentration of one to three microcuries per ml. To carry out longer experiments, the isotope concentration in food must be correspondingly increased.

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